23

Using dairy ingredients to produce edible films and biodegradable packaging materials

P. M. Tomasula, United States Department of Agriculture, USA

Abstract: Major food retailers and consumers are concerned about the waste that packaging generates and the scarce natural resources and energy used in its manufacture. Edible films and coatings made from food-grade proteins and carbohydrates are an untapped source of renewable material that, while compostable and biodegradable, can also be consumed with the food product. This chapter focuses on films and coatings made from dairy proteins with an emphasis on those based on casein and whey, the major proteins found in milk, and the research efforts that have been undertaken to improve their mechanical and barrier properties.

Key words: edible films and coatings, packaging, renewable materials, dairy proteins, casein, whey, barrier properties.

23.1 Introduction

23.1.1 Food packaging films

In an effort to reduce food packaging waste, manufacturers are turning their attention to renewable materials to replace petroleum-based packaging films. Edible films and coatings made from food-grade proteins and carbohydrates are an untapped source of renewable material that, while compostable and biodegradable, can also be consumed with the food product. This chapter focuses on edible films and coatings made from dairy proteins with an emphasis on those based on casein and whey, the major proteins found in milk, and the research efforts that have been undertaken to improve their mechanical and barrier properties so that they may be used in a variety of applications.

23.1.2 Edible films and coatings

Edible protein-based *films* are usually formed as free-standing thin sheets, while edible *coatings* are thin films formed directly on the food product. Edible films have the potential for use as food wrapping, or as part of the food itself, to extend its shelf-life and enhance its properties. Edible films and coatings also have the potential to replace one or more polymeric film layers in multilayer packaging systems, being placed next to the food to protect it from contact with the polymeric packaging or to facilitate recycling.

In edible films and coatings, the protein functions as the foundation and matrix of the film. Most films or coatings made from proteins are strong, but are brittle and not crease resistant, have limited ability to elongate or stretch, and swell or dissolve under humid conditions. Most are excellent oxygen barriers.

Besides being biodegradable and compostable, edible films or coatings based on proteins are desirable because they also offer a lucrative outlet for surplus agricultural materials. Edible films or coatings may be used to inhibit migration between components in foods, to evenly distribute food ingredients and additives throughout a food product, to modify the appearance of foods, and to improve the mechanical integrity and handling characteristics of foods. They also may be used as mass transfer barriers between the food and the environment, controlling transfer of moisture, oxygen, carbon dioxide, and aromas (Kester and Fennema, 1986). Edible films and coatings are not a replacement for synthetic packaging materials for prolonged storage of food but are to be used to improve food quality, extend shelf-life and possibly improve the efficiency of packaging materials (Kester and Fennema, 1986). The films may also carry antimicrobials and be used at the surface of foods to prevent bacterial growth. They may also be formed into pouches or bags for individual or small portions of food, or used for microencapsulation of food ingredients to control their addition and release into foods (Robertson, 1993).

23.2 The milk proteins used for film formation

23.2.1 Casein and caseinate films

Casein and whey are the major milk proteins, with casein comprising approximately 80% of the milk proteins. Casein exists as a micelle in milk and is comprised of the amphipathic αs_1-, αs_2-, β- and κ-caseins in the ratio of 40:10:35:12. The caseins are bound by calcium-phosphate linkages. αs_1-, αs_2- and β-caseins are within the hydrophobic core of the micelle and are calcium sensitive, while κ-casein is distributed over the micelle, stabilizing it through steric stabilization.

αs_1- and β-casein do not contain cysteine residues and, unlike the whey proteins, the caseins do not form disulfide bonds. Because of a large number

of proline residues, casein assumes a random-coil structure. Its loose, open structure, which exposes the hydrophobic groups, is sensitive to environmental changes, such as pH changes or changes in temperature, which are exploited to precipitate casein from milk.

Casein may be precipitated from milk by (i) enzyme treatment with chymosin or rennet, as in cheese making, to make para-κ-casein, more popularly known as rennet casein; (ii) through the action of lactic acid bacteria, to make lactic acid casein; or (iii) by addition of acids to make acid casein. In rennet casein production, rennet cleaves the Phe (105)-Met (106) linkage of κ-casein, destabilizing the micelle. Para-κ-casein remains with the micelle and is hydrophobic. The rest of the peptide, known as casein macropeptide, is hydrophilic and is no longer part of the micelle. The casein micelles aggregate due to hydrophobic interactions and are usually heated to about 40°C to firm the precipitated curd. The calcium (Ca) content of the resulting curd ranges from 2.6 to 3.0% and the pH of the curd is 7.3 to 7.7 (Southward and Walker, 1980). Rennet casein is insoluble in water.

Acid casein may be produced either through the addition of lactic acid bacteria, which lowers the pH of milk to 4.6 after an incubation period of 14 to 16 hours (Southward and Walker, 1980), or by the addition of a mineral acid such as HCl or H_2SO_4 until the pH of milk is 4.6. As pH of milk is lowered from pH 6.7 to 5.4 during acid casein production, the internal Ca-phosphate linkages stabilizing the micelle are solubilized into the whey. Destabilization of the micelle begins. As pH is decreased further, the surface of the micelle, which is negative, is neutralized and aggregation begins because of hydrophobic interactions. The resulting acid curd is heated to 40°C to firm the curd. The Ca content of acid casein is approximately 0.1% (Southward and Walker, 1980). Acid caseins are insoluble in water.

Because acid and rennet caseins are insoluble in water, they are converted to water soluble forms to make films. Acid caseins are reacted with bases to form the water soluble caseinates. The most common caseinates are calcium caseinates (CaCAS) and sodium caseinates (NaCAS), which are the products of the reaction of acid casein with either $Ca(OH)_2$ or NaOH, respectively. The micellar structure of casein is not restored upon addition of the base. The Ca content of CaCAS is approximately 1.3% and the Na content of NaCAS ranges from approximately 1.2 to 1.4%. The random coil nature of the caseinates and their ability to form hydrogen, electrostatic and hydrophobic bonds makes them excellent film and coating formers (Avena-Bustillos and Krochta, 1993). The caseinates show good adhesion to different substrates due to their high amount of polar groups and their hydrophilicity makes them excellent barriers to substances such as oxygen, carbon dioxide, and aromas (Audic *et al.*, 2003). However, their hydrophilic nature makes them a weak barrier to moisture. Much of the research on edible casein films to date is directed at improving their water

vapor barrier properties. The caseinates can also participate in enzymatic and chemical reactions to modify their properties for food or nonfood use (Li Chan, 2004; Audic *et al.*, 2003; Santos *et al.*, 1999; Santos and Tomasula, 2000) but these types of modifications have rarely been explored to modify the properties of edible films. Also, the film forming properties of the individual caseins such as αs1-casein and β-casein, which are less commercially available, have been studied to a very limited extent.

In a novel process (Jordan *et al.*, 1987; Tomasula *et al.*, 1995), casein has also been precipitated from milk using high-pressure carbon dioxide (CO_2). Addition of CO_2 to milk causes carbonic acid to form, which in turn, lowers the pH of the milk. With CO_2 pressures greater than 5.2 MPa at 38°C, the pH is reduced to about 5.4 and CO_2–casein (CO_2–CAS) precipitates. The pH of the curd ranges from 5.4 to 5.8, varying with CO_2 pressure. CO_2–CAS has some of its micellar structure intact, which imparts a haze to films made from this casein. A continuous process (Tomasula *et al.*, 1997) was also developed to produce CO_2–CAS. It has a Ca content of approximately 1%, is 7% soluble in water and its other properties are between those of acid and rennet casein. The performance of the CO_2–CAS films though is similar to that of CaCas films.

23.2.2 Whey protein films

Sweet whey, once considered a waste of the cheese making process, is now an important product of cheese manufacture. Using technologies such as ultrafiltration and ion exchange, whey protein concentrates (WPC) with protein contents ranging from 35 to 85% and whey protein isolates (WPI) with protein contents greater than 90% are commercially available (Bonnaillie and Tomasula, 2008). Whey protein-based edible films are usually made from commercial WPI, though some have been made using WPC. Commercial WPI, depending on manufacturing process, is comprised of approximately 30% alpha-lactalbumin (α-LA) and 60% beta-lactoglobulin (β-LG), 7% of other whey proteins, and small amounts of lactose, ash and fat. WPI is chosen over other whey products in order to exploit the gelling and solubility properties of β-LG (Kilara and Vaghela, 2004).

The whey proteins are globular proteins, soluble over a wide range of pH, and are denatured by heat. Unlike casein, the hydrophobic, polar, and charged amino acids are uniformly distributed over the whey protein. The proteins fold so that most of the hydrophobic groups are buried inside the structure. At temperatures between 67 and 82°C and pH of 7.5 (the conditions important for making water insoluble whey protein films), the globular structure of β-LG opens, exposing the free thiol group at CYS 121 (Dangaran and Krochta, 2008) and the hydrophobic groups. Polymerization then occurs through intermolecular disulfide bonding by thiol – disulfide interchange and thiol oxidation reactions.

23.3 Edible films and coatings made from casein or whey proteins

23.3.1 Edible film and coatings preparation

Edible films and coatings are formed by simple coacervation, complex coacervation, or by thermal gelation or precipitation (Kester and Fennema, 1986). The performance of the films in an application depends on the conformation of proteins in the film, which is influenced by the composition of the film, the solvent environment used to dissolve the protein, the influence of the ambient environment, and the techniques used to dry and process the films. Post-treatment of the films to modify surface texture is also used to modify the properties of the films.

For edible film and coating applications, the proteins are dissolved in either water or food-grade ethanol or mixtures of the two solvents and stirred or homogenized. The solution is placed under mild vacuum to remove dissolved air. There are several methods available for preparing edible films, and solvent casting is the one mostly used in laboratories. In solvent casting, a weighed amount of the film solution, usually containing about 2 to 10% (w/w) of protein, is pipetted into a polystyrene or PTFE petri dish, or spread onto any other flat surface or substrate that can contain the solution. The film preparation is then allowed to dry overnight under ambient conditions. The dried film is then peeled from the dish or other surface and is stored at 50% relative humidity (RH) in a dessicator at room temperature. Thickness is measured using a micrometer at up to 10 points across the film to determine an average film thickness. Film thickness for a particular protein may be varied by varying the amount of solids in the film-forming solution. For edible coatings, the protein solution is sprayed directly onto the food, onto other films, or used as a dipping solution.

Kozempel *et al.* (2003) determined that the optimal concentration for preparing edible films made from CaCAS or CO_2–CAS was 10% (w/w). Surface tension, a measure of the inward forces that must be overcome to expand the surface area of a liquid (Whitten *et al.*, 1992), was essentially constant for solution concentrations in the range from 4 to 10% (w/w), then increased rapidly with concentrations greater than 10% (w/w). Above 10% solids, the high viscosity of the protein solution made it difficult to remove air bubbles before casting films and entrapped bubbles, and other defects were noted in the dried films.

The choice of substrate is also important for film casting. The film solution must uniformly spread over, or wet, the substrate and be readily released when the film is dry (Churaev, 2003; Kozempel and Tomasula, 2004). The substrate is an environmental factor that influences the properties of the finished film; film defects and properties may be attributed to the temperature and type of substrate (Kester and Fennema, 1986).

Few studies have examined the effects of drying techniques or parameters on the barrier and mechanical properties of films. Drying techniques

may also affect the appearance of the films (Perez-Gago and Krochta, 2000). The kinetic mechanism for ambient air-drying of films or drying of films in a hot-air dryer proceeds in three-stages (Kozempel *et al.*, 2003). The first drying stage is a constant rate period in which surface evaporation is the rate-controlling step. The second drying stage is a falling rate period in which the solvent can no longer move to the surface of the film to saturate the surface and diffusion begins to predominate as the rate-controlling step. The third stage is a second falling rate period that is unlikely to be observed graphically for thin edible films. At this stage, the evaporating surface of the film is located deeper within the film and the film itself restricts diffusion. For hot air drying of caseinate films, the optimal drying temperature for clear colorless films was 34°C. Higher temperatures led to slightly yellow and progressively darker films.

Cast films tend to be shiny on the side that faced the substrate and matte on the side facing the air. The barrier properties of the films appear to be independent of the side facing the highest concentration of permeant, with the exception of emulsion films as discussed below.

23.3.2 Plasticizers

Protein films tend to shrink and become brittle as they dry, making removal of a single intact film from the substrate difficult. Addition of a plasticizer, a low molecular weight compound, is required to impart flexibility to the films. Plasticizers reduce the internal hydrogen bonding and electrostatic interactions that operate within the protein chains and reduce the glass transition temperature (Krochta and De Mulder-Johnson, 1997). They also increase the molecular spacing in films or, in the case of low molecular weight compounds, occupy the free volume. Proteins tend to have a large free volume relative to that of synthetic polymers because they are less linear with many amino acid side groups. Internal water also acts as a plasticizer and its effects may be difficult to control because protein films are sensitive to the RH of the environment (Coupland *et al.*, 2000).

Various food-grade plasticizers have been used to impart flexibility and elongation to edible films. Polyols such as glycerol (GLY), which is hydrophilic, are added to the protein solution before solvent casting or other types of processing. GLY may also establish hydrogen bonding with amino acid residues of casein (Tomasula *et al.*, 1998) and electrostatic attraction between calcium and the hydroxyl groups of GLY is possible. Other plasticizers that have been used in edible films are propylene glycol (PG), polypropylene glycol (PPG), sorbitol (SOR), sucrose and various blends of the polyols.

The choice of a plasticizer depends on its size and shape as well as its compatibility with the protein. While plasticizers increase the flexibility of the films, they modify their barrier and mechanical properties by decreasing the strength of the films while increasing their permeability. Dangaran and

Krochta (2007) demonstrated that, over time, the plasticizer itself may crystallize, as is the case with sucrose, and affect the properties of the film. Addition of a crystallization inhibitor such as raffinose or lactose is necessary to prevent this change in the plasticizer.

23.3.3 Barrier and mechanical properties of films

Water vapor permeability (WVP) and oxygen permeability (OP) are the most commonly reported barrier properties for edible films. The mechanism by which water vapor or a gas such as oxygen permeates a protein film is assumed to occur as follows: the permeant is absorbed into the film matrix at the high concentration side, dissolves and diffuses into the film according to the concentration gradient, and is desorbed and evaporates from the other side of the film. Dissolution and diffusion of the permeant depends on the structure of the protein film matrix and the properties of the permeant. The structural aspects of the film such as its hydrogen bonding, van der Waals interactions, degree of cross-linking, and crystallinity, as well as the amount and type of plasticizer have been implicated (Kumins, 1965; Siew *et al.*, 1999; Coupland *et al.*, 2000). The properties of the permeant such as its temperature, size, shape and polarity and its solubility in the film are also of importance (Kester and Fennema, 1986). Because of the many variables affecting barrier properties of edible films, reporting of their properties requires that the thickness of the films, RH gradient across the films, and temperature be reported as well.

For protein films, permeability is the product of the diffusion coefficient and the solubility, and is dependant on the cross-sectional area of the film, A, and the thickness of the film, L. The permeability, P, is defined by the following equation (Kester and Fennema, 1986) based on Fick's First Law of Diffusion and Henry's Law of Solubility:

$$P = DS = QL/(At\Delta p) \qquad [23.1]$$

where D is the diffusion coefficient, S is the solubility coefficient, Q is the quantity of permeant passing through the film, and Δp is the partial pressure difference across the film. This equation assumes that D and S are independent of the concentration of the permeant. For hydrophilic protein films, the water sorption isotherms are nonlinear and anomalous effects in film properties have been observed compared to polymer-based films. For example, WVP has been shown to vary with the thickness of NaCAS films but is a constant value for polymer films. McHugh *et al.* (1993) demonstrated that WVP increases with increasing film thickness because of the associated increase in RH of these films as the absorbed water vapor interacts with the hydrophilic, polar films and imparts a plasticizing effect. The amount of hydrophilic plasticizer incorporated into the film is also responsible for increases in the water vapor permeability.

A model was developed by Buonocore *et al.* (2003) to gain insight into the phenomena controlling water solubilization and diffusion into

casein–starch-based edible coatings in which solubility and diffusion processes were described separately. The model successfully fitted the experimental data.

Water Vapor Permeability

Standard E96-95 (ASTM, 1995) is usually applied for the determination of WVP of films although instrument methods are also available. Most researchers apply the correction factor of McHugh *et al.* (1993) for hydrophilic films, which accounts for the water vapor partial pressure gradient in the stagnant air layer of the test cups used for testing. The WVP of several milk-based films are reported in Table 23.1. In general, WVP of milk protein films are several times greater than those of synthetic films.

A comparison of the WVP of films based on the type of protein or plasticizer used is difficult because of the variation in experimental conditions employed to test the films and the variations in film thicknesses. Lower WVP are apparent for the thinner films as is the case for the CaCAS:GLY and CO_2–CAS:GLY films (Tomasula *et al.*, 1998) and WVP increases with increasing film thickness. However, the increase noted in the case of WPI films plasticized with GLY is probably insignificant (Longares *et al.*, 2004).

Instead of adding plasticizer to films to reduce the intermolecular forces between protein chains, Sothornvit and Krochta (2000b) hypothesized that using WPI with some degree of hydrolysis would increase the number of polymer chain end groups and the free volume and, as a consequence, reduce the amount of plasticizer needed. It was shown that the whey protein molecular weight had little effect on WVP, and that decreasing the molecular weight increased the solubility of the films and improved their flexibility relative to WPI films.

Oxygen permeability

Most milk protein films are excellent oxygen barriers, especially at low RH, compared to synthetic films such as low density polyethylene (LDPE) and high density polyethylene (HDPE) and approach or equal the properties of films such as ethylene vinyl alcohol (EVOH) and poly(vinylidene chloride) (PVDC) due to their high degree of hydrogen bonding (McHugh and Krochta, 1994b). Even though milk protein films have relatively high WVP which would prevent their use in many applications, their low oxygen permeabilities (OP) make them excellent candidates to consider for a particular oxygen barrier application or to use as part of a composite system.

Instruments are available for the determination of OP over wide ranges of temperature, typically from 10 to 40°C and RH at 0 and from 30 to 75%. The instruments operate according to Standard Method D-3985-95 (ASTM, 1995a). A sample of the film is clamped into a diffusion cell, is purged using pure oxygen, and then pure oxygen is introduced to the cell. Molecules of oxygen that diffuse through the film are then sensed by an oxygen sensor. RH probes are also located on both sides of the film for control of RH.

Table 23.1 Water vapor permeability (WVP) properties of solvent cast protein films and synthetic films

Dairy protein	Composition	Weight ratio (protein/ plasticizer)	Thickness (mm)	Test conditions T°C; RH %*	WVP g.mm/ m^2.h.KPa	Reference
NFDM	NFDM:potassium sorbate	9:1	0.110	25; 0/74	3.32	Maynes & Krochta (1994)
	NFDM:hydrolyzed lactose	–	0.120	25; 0/72	3.76	Maynes & Krochta (1994)
	NFDM (ultrafiltered):GLY	4:1	0.071	25; 0/65	2.93	Maynes & Krochta (1994)
β-casein	β-casein:GLY	2:1	0.11–0.12	22.5; 53/11	0.654	Mauer *et al.* (2000)
Lactic acid casein	LAC:GLY	0.6:1	0.203	37.8; 90	2.29	Chick & Ustunol (1998)
	LAC:GLY	1:1	0.203	37.8; 90	2.47	Chick & Ustunol (1998)
	LAC:GLY	1.4:1	0.203	37.8; 90	2.28	Chick & Ustunol (1998)
	LAC:SOR	0.6:1	0.203	37.8; 90	1.87	Chick & Ustunol (1998)
	LAC:SOR	1:1	0.203	37.8; 90	1.88	Chick & Ustunol (1998)
	LAC:SOR	1.4:1	0.203	37.8; 90	1.42	Chick & Ustunol (1998)
	LAC:SOR	1:1	0.104	37.8; 50	0.081	Chick & Hernandez (2002)
	LAC:SOR	1:1	0.104	37.8; 70	0.667	Chick & Hernandez (2002)
	LAC:SOR:carnauba wax	3.33:2.33:1	0.104	37.8; 50	0.039	Chick & Hernandez (2002)
	LAC:SOR:carnauba wax	3.33:2.33:1	0.104	37.8; 70	0.495	Chick & Hernandez (2002)
	LAC:SOR:candelilla wax	3.33:2.33:1	0.104	37.8; 50	0.025	Chick & Hernandez (2002)
	LAC:SOR:candelilla wax	3.33:2.33:1	0.104	37.8; 70	0.185	Chick & Hernandez (2002)
Rennet casein	REN:GLY	0.6:1	0.203	37.8/90	2.41	Chick & Ustunol (1998)
	REN:GLY	1:1	0.203	37.8/90	2.43	Chick & Ustunol (1998)
	REN:GLY	1.4:1	0.203	37.8/90	1.88	Chick & Ustunol (1998)
	REN:GLY	4:1	0.091	25; 0/77	2.33	McHugh & Krochta (1994d)
	REN:SOR	0.6:1	0.203	37.8/90	2.07	Chick & Ustunol (1998)
	REN:SOR	1:1	0.203	37.8/90	2.07	Chick & Ustunol (1998)
	REN:SOR	1.4:1	0.203	37.8/90	1.65	Chick & Ustunol (1998)
MgCAS	MgCAS:GLY	4:1	0.077	25; 0/77	1.83	McHugh & Krochta (1994d)

Table 23.1 *Cont'd*

Dairy protein	Composition	Weight ratio (protein/ plasticizer)	Thickness (mm)	Test conditions T°C; RH %*	WVP g.mm/ m^2.h.KPa	Reference
Calcium caseinate	CaCAS	–	0.082	25; 0/85	1.17	Avena-Bustillos & Krochta (1993)
	CaCAS:BW	1.7:1	0.075	25; 0/97	0.15	Avena-Bustillos & Krochta (1993)
	CaCAS:GLY	2:1	0.105	23; 0/72	7.9	Banerjee & Chen (1995)
	CaCAS:GLY	2.33:1	0.171	30; 0/87	3.18	Tomasula *et al.* (1998)
	CaCAS:GLY	2.33:1	0.222	30; 0/86	4.45	Tomasula *et al.* (1998)
	CaCAS:GLY	2.33:1	0.100	30; 0/85	2.40	Tomasula *et al.* (2003)
	CaCAS:GLY	9:1	0.331	25; 0/82	3.54	Dangaran *et al.* (2006)
	CaCAS:GLY	4:1	0.301	25; 0/82	5.04	Dangaran *et al.* (2006)
	CaCAS:GLY	2.33:1	0.280	25; 0/82	6.69	Dangaran *et al.* (2006)
	CaCAS:GLY:PPG	2.33:0.75:0.25	0.100	30; 0/85	2.00	Tomasula *et al.* (2003)
CO_2 casein	CO_2–CAS:GLY	2.33:1	0.112	30; 0/86	2.22	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.163	30; 0/88	2.58	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.184	30; 0/88	3.21	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.277	30; 0/90	3.80	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.100	30; 0/89	2.30	Tomasula *et al.* (2003)
	CO_2–CAS:GLY (Particle size = 111.8 μm)	9:1	0.236	25; 0/82	2.32	Dangaran *et al.* (2006)
	CO_2–CAS:GLY (Particle size = 111.8 μm)	4:1	0.229	25; 0/82	3.47	Dangaran *et al.* (2006)
	CO_2–CAS:GLY (Particle size = 111.8 μm)	2.33:1	0.100	25; 0/82	3.57	Dangaran *et al.* (2006)
	CO_2–CAS:GLY:PPG	2.33:0.75:0.25	0.100	30; 0/89	1.90	Tomasula *et al.* (2003)

AcCAS	Acylated casein	2.33:1	0.175	30; 0/90	2.40	Tomasula *et al.* (2003)
Sodium caseinate	NaCAS	–	0.083	25; 0/81	1.53	Aveno-Bustillos & Krochta (1993)
	NaCAS	–	0.3364	23; 0/90	1.9	Schou *et al.* (2005)
	NaCAS (Buffer-treated pH 4.6 with Ca Ascorbate)	–	0.072	25; 0/86	0.87	Aveno-Bustillos & Krochta (1993)
	NaCAS:GLY	2:1	0.109	23; 0/55	12.9	Banerjee *et al.* (1996)
	NaCAS:GLY	0.89:1	0.104	20; 45/0	0.473	Siew *et al.* (1999)
	NaCAS:GLY	1.67:1	0.072	20; 45/0	0.225	Siew *et al.* (1999)
	NaCAS:GLY	2.125:1	0.3577	23; 0/90	3.0	Schou *et al.* (2005)
	NaCAS:GLY	4:1	0.10	20; 0/90	0.303	Khwaldia *et al.* (2004a)
	NaCAS:GLY	4:1	0.065	25; 75	0.25	Chambi & Grosso (2006)
	NaCAS:PEG	0.81:1	0.099	20; 45/0	0.936	Siew *et al.* (1999)
	NaCAS:PEG	1.32:1	0.071	20; 45/0	0.612	Siew *et al.* (1999)
	NaCAS:Lauric Acid	4:1	0.074	25; 0/92	0.40	McHugh & Krochta (1994d)
	NaCAS:AM	1:4	0.04	25; 0/84	0.66	Aveno-Bustillos & Krochta (1993)
	NaCAS:AM	1:1	0.088	25; 0/88	0.92	Avena-Bustillos & Krochta (1993)
	NaCAS:BW	1.66:1	0.103	25; 0/95	0.40	Avena-Bustillos & Krochta (1993)
	NaCAS:GLY:AMF	10:2.5:1	0.100	20; 0/90	0.300	Khwaldia *et al.* (2004a)
	NaCAS:GLY:AMF	3.33:0.83:1	0.100	20; 0/90	0.506	Khwaldia *et al.* (2004a)
	NaCAS:Gelatin:GLY	3:1:1	0.065	25; 0/75	0.29	Chambi & Grosso (2006)
	w/transglutaminase	3:1:1	0.065	25; 0/75	0.21	Chambi & Grosso (2006)

Table 23.1 *Cont'd*

Dairy protein	Composition	Weight ratio (protein/ plasticizer)	Thickness (mm)	Test conditions T°C; RH %*	WVP g.mm/ m^2.h.KPa	Reference
Whey protein isolate	Native WPI:GLY	2.33:1	0.139	25; 0/71	5.06	Perez-Gago *et al.* (1999)
	WPI:GLY	2.33:1	0.139	25; 0/71	4.96	Perez-Gago *et al.* (1999)
	WPI:GLY	1.6:1	0.106	25; 0/11	0.275	McHugh *et al.* (1994)
	WPI:GLY	1.6:1	0.121	25; 0/65	5.00	McHugh *et al.* (1994)
	WPI:GLY	4:1	0.129	25; 0/77	2.93	McHugh *et al.* (1994)
	WPI:GLY	15:1	0.11–0.18	26.3; 0/88	1.8918	Shellhammer & Krochta (1997a)
	WPI:SOR	1.6:1	0.13	25; 0/79	2.58	McHugh *et al.* (1994)
	WPI:BW:SOR (Mean Diameter = 1.97 μm)	3.5:1.8:1	0.19	25; 0/93	1.17	McHugh & Krochta (1994a)
	WPI:BW:SOR (Mean Diameter = 0.82 μm)	3.5:1.8:1	0.14	25; 0/98	0.221	McHugh & Krochta (1994a)
	Hydrolyzed WPI (5.5%):GLY	2.33:1	–	25; 0/90	4.2	Sothornvit & Krochta (2000b)
	Hydrolyzed WPI (10%):GLY	2.33:1	–	25; 0/90	4	Sothornvit & Krochta (2000b)
Synthetic films	HDPE	–	–	38; 90/0	.00083	McHugh & Krochta (1994d)
	LDPE	–	–	27.6; 0/100	.0013	Shellhammer & Krochta (1997)
	PVDC	–	–	27.6; 0/100	.0008	Shellhammer & Krochta (1997)
	PVC	–	–	27.6;0/100	0.0257	Shellhammer & Krochta (1997)
	Beeswax	–	–	25.9; 0/100	.0037	Shellhammer & Krochta (1997)
	Anhydrous Milkfat	–	–	27.5;0/100	0.5993	Shellhammer & Krochta (1997)
	Carnauba Wax	–	–	27.5;0/100	0.0041	Shellhammer & Krochta (1997)
	Candelilla Wax	–	–	24.9;0/100	0.0005	Shellhammer & Krochta (1997a)

* %RH = relative humidities outside and inside of test cup if used, (outside/inside), otherwise, a single RH% is reported.
NFDM = nonfat dry milk; GLY = glycerol; SOR = sorbitol; BW = beeswax; PPG = polypropylene glycol; AM = acetylated monoglyceride; PEG = polyethylene glycol; MgCas = Magnesium caseinate; AMF = anhydrous milk fat; LDPE = low density polyethylene; HDPE = high density polyethylene; PVDC = poly(vinylidene chloride); PVC = poly(vinyl chloride).

The OP of various milk protein films are listed in Table 23.2 along with those of synthetic films for comparison. The OP is a function of plasticizer content, type of protein, RH and temperature. Examination of the table shows that for any given milk protein, films plasticized with SOR, which is a solid at room temperature, are more effective oxygen barriers than films plasticized with GLY. The improved OP of the films plasticized with SOR may be due to the creation of impenetrable crystalline domains within the film that lower permeability, although it was expected that the crystalline domains would negatively affect the tensile properties (Dangaran and Krochta, 2008). The OP of films increased with increasing amounts of hydrophilic plasticizer.

The protein type also affects the oxygen barrier properties of the films. Lactic acid casein and rennet films had lower OP but were tested under different conditions from many of the other films. For films tested at 23°C and 50% RH, CaCAS and WPI plasticized with 30% GLY have OP of 86 and 76.1, respectively, but the OP of CO_2–casein is 144 cc-μm/KPa.d.m^2, almost twice as large. OP is related to the aggregation of the protein, its conformation due to environmental variables, and its impact on the free volume.

Mechanical properties

The mechanical properties of milk-based films are usually reported in terms of the tensile strength (TS), a measure of the strength of the film, and percent elongation (%E), a measure of the distance that the film will stretch from its initial length before breaking, or of its flexibility. The tensile properties are measured according to Standard D882-01 (ASTM, 2001) for plastic films. Prior to measurement of TS, the milk protein based films are conditioned at 23°C and 50% RH for at least 40 hours. Overall, the milk protein films are strong due to the extensive intermolecular forces operating among the protein chains, but plasticizers reduce these forces and increase protein chain mobility, which improves the flexibility of the films. The tensile properties of milk-based films are reported in Table 23.3 and tend to vary according to types of protein and plasticizer used and film thickness.

In general, the TS values for most films approach those of the synthetic films but %E are much lower. Higher TS values of films tend to correlate with greater protein content. Higher %E values are desirable because the films are more durable when handled during processing or during use by the consumer. Addition of plasticizer weakens the milk-based films but at the lowest values of plasticizer, 10–15% GLY, the milk protein based films are approximately as strong as the LDPE and HDPE films. Increasing GLY plasticizer weakens the films further but improves the %E. Whey and caseinate films plasticized with SOR are approximately as strong as those plasticized with GLY but exhibit lower %E because the crystalline structure of SOR inhibits flexibility.

Table 23.2 Oxygen permeabilities of solvent cast protein films compared with synthetic films. Test conditions were 23°C and 50% RH unless otherwise noted

Dairy protein	Composition	Weight ratio (protein/ plasticizer)	Thickness (mm)	Oxygen permeability (cc. μm/kPa.d.m^2)	Reference
Lactic acid casein	LAC:GLY[a]	0.6:1	0.203	0.88	Chick and Ustunol (1998)
	LAC:GLY[a]	1:1	0.203	2.18	Chick and Ustunol (1998)
	LAC:GLY[a]	1.4:1	0.203	0.73	Chick and Ustunol (1998)
	LAC:SOR[a]	0.6:1	0.203	0.65	Chick and Ustunol (1998)
	LAC:SOR[a]	1:1	0.203	0.73	Chick and Ustunol (1998)
	LAC:SOR[a]	1.4:1	0.203	0.81	Chick and Ustunol (1998)
	LAC:SOR	1:1	0.104	0.414	Chick and Hernandez (2002)
	LAC:SOR:carnauba wax	3.33:2.33:1	0.104	0.544	Chick and Hernandez (2002)
	LAC:SOR:candelilla wax	3.33:2.33:1	0.104	0.613	Chick and Hernandez (2002)
Rennet casein	REN:GLY[a]	0.6:1	0.203	7.06	Chick and Ustunol (1998)
	REN:GLY[a]	1:1	0.203	5.55	Chick and Ustunol (1998)
	REN:GLY[a]	1.4:1	0.203	1.84	Chick and Ustunol (1998)
	REN:SOR[a]	0.6:1	0.203	0.71	Chick and Ustunol (1998)
	REN:SOR[a]	1:1	0.203	1.02	Chick and Ustunol (1998)
	REN:SOR[a]	1.4:1	0.203	0.96	Chick and Ustunol (1998)
Calcium caseinate	CaCAS:GLY	2.33:1	0.1	86	Tomasula *et al.* (2003)
	CaCAS:GLY:PPG	2.3:0.75:0.25	0.1	68	Tomasula *et al.* (2003)
Sodium caseinate	NaCas:GLY	4:1	0.100	44.6	Khwaldia *et al.* (2004a)
	NaCas:GLY:AMF	10:2.5:1	0.100	38.4	Khwaldia *et al.* (2004a)
	NaCas:GLY:AMF	3.33:0.83:1	0.100	33.6	Khwaldia *et al.* (2004a)

CO_2–casein	CO_2–CAS:GLY	2.3:1	0.1	144	Tomasula *et al.* (2003)
	CO_2–CAS:GLY:PPG	2.3:0.75:0.25	0.1	74	Tomasula *et al.* (2003)
AcCas	Acylated casein	2.33:1	0.175	48	Tomasula *et al.* (2003)
Whey protein isolate	Hydrolyzed WPI:GLY 5.5% DH	3.1–1.8:1	0.0130	42.2–111.9	Sothornvit and Krochta (2000a)
	Hydrolyzed WPI:GLY 10% DH	3.1–1.8:1	0.0130	35.5–89.1	Sothornvit and Krochta (2000a)
	Unhydrolyzed WPI:GLY	3.1–0.8:1	0.013	41.3–333.1	Sothornvit and Krochta (2000a)
	WPI:GLY	2.3:1	0.11	76.1	McHugh and Krochta (1994b)
	WPI:GLY	5.7:1	0.11	18.5	McHugh and Krochta (1994b)
	WPI:SOR	2.3:1	0.11	4.3	McHugh and Krochta (1994b)
	WPI:SOR	1:1	0.11	8.3	McHugh and Krochta (1994b)
	WPI:SOR	3.5:1	0.11	2.6	McHugh and Krochta (1994b)
	WPI:SOR[b]	3.5:1	0.11	0.7	McHugh and Krochta (1994b)
	WPI:SOR[c]	3.5:1	0.11	43.3	McHugh and Krochta (1994b)
Synthetic films	LDPE	–	0.0254	1870	Salame (1986)
	HDPE	–	–	427	Salame (1986)
	PVDC-based films	–	–	0.38–5.1	Salame (1986)
	EVOH (70% VOH)[d]	–	–	12	Salame (1986)

[a] Test conditions, 23°C, 0% RH; [b] test conditions 23°C, 40% RH; [c] test conditions 23°C, 70% RH; [d] test conditions 23°C, 95% RH.
GLY = glycerol; SOR = sorbitol; PPG = polypropylene glycol; AMF = anhydrous milk fat; LDPE = low density polyethylene; HDPE = high density polyethylene; PVDC = poly(vinylidene chloride).

Tensile properties are difficult to determine for milk protein films without added plasticizer because of their brittleness; however, Schou *et al.* (2005) report TS for a NaCAS film without added plasticizer which surpasses that of LDPE and HDPE films. On the other hand, %E of the NaCAS film is only 2.1% compared to values of 500 and 300%, respectively, for the LDPE and HDPE films.

The lactic acid and rennet casein films (Chick and Ustunol, 1998), which were made by adjusting pH to 10 using NaOH to solubilize the caseins in water prior to film making, were plasticized with greater amounts of GLY and SOR than for the other films reported in Table 23.3. The TS of these films plasticized with about 40% GLY are about as strong as the whey and casein films plasticized with 30% GLY yet have %E approaching that of the synthetic films, possibly due to the greater plasticizer content.

Little information is available on the performance of individual caseins in films. Mauer *et al.* (2000) showed that films made from the most hydrophobic component of casein, β-casein, demonstrated marginal improvement in WVP and TS, but % E was about 250% compared to approximately 80% obtained for both CO_2–CAS and CaCAS films (Tomasula *et al.*, 1998). Motoki *et al.* (1987) made films from the hydrophilic α_{s1}-casein cross-linked with transglutaminase. The resulting films were water insoluble but the tensile properties were comparable to those of CO_2–CAS or CaCAS films.

Rapid drying of solvent cast films may limit the mobility of the protein chains, preventing the development of intermolecular interactions in films as the solvent is removed (Banker, 1966). This was demonstrated by Alcantara *et al.* (1998) for the drying of whey protein isolate films. Water vapor permeabilities were lower for films dried at 95°C and 30% RH than for films dried at 21°C and 50% RH. The films dried at 95°C were stronger but less extensible than films dried at 21°C. Kaya and Kaya (2000) examined the effects of microwave drying on the properties of whey protein isolate films containing 50% WPI and 50% GLY. Drying time was reduced to 5 minutes compared to 18 hours of drying under room conditions. TS and %E improved by approximately 15 and 40%, respectively, with microwave drying but WVP did not improve.

Aroma barrier properties and appearance of milk protein films

Information on the aroma barrier properties of casein films is scarce but there are a few studies that report the aroma barrier properties of whey protein films, such as those reported in Dangaran and Krochta (2008).

Caseinates and whey proteins form films that are transparent and glossy. These characteristics are important for using the films as coatings or in laminates with synthetic films. CO_2–CAS films (Tomasula *et al.*, 1998) are nearly opaque and hazy because of the partial micellar character of the films. Standards D523-89 (ASTM, 1989) and D4039-93 (ASTM, 1999) are used to measure gloss and haze of films. Dangaran *et al.* (2006) improved the gloss of CO_2–CAS films from 55.3 gloss units to 73 gloss units by

Table 23.3 Tensile properties of protein films compared with synthetic films. Test conditions were 23°C and 50% RH unless otherwise noted

Dairy protein	Composition	Weight ratio (protein/plasticizer)	Thickness (mm)	Tensile strength (MPa)	Percent elongation (%)	References
NFDM	NFDM (Lactose free):GLY	4:1	0.069	5.1	12.2	Maynes and Krochta (1994)
	NFDM (ultrafiltered):GLY	4:1	0.071	10.0	5.2	Maynes and Krochta (1994)
α_{s1}-casein	α_{s1}-casein:GLY	49:1		4.1	38	Motoki *et al.* (1987)
	α_{s1}-casein:GLY w/ transglutaminase	49:1		10.6	77	Motoki *et al.* (1987)
β-casein	Beta-casein:GLY	2:1	0.11–0.12	6.0	274	Mauer *et al.* (2000)
Lactic acid casein	LAC:GLY	0.6:1	0.216	0.42	121.4	Chick and Ustunol (1998)
	LAC:GLY	1:1	0.216	1.24	253.6	Chick and Ustunol (1998)
	LAC:GLY	1.4:1	0.216	2.51	194.1	Chick and Ustunol (1998)
	LAC:SOR	0.6:1	0.216	2.43	170.7	Chick and Ustunol (1998)
	LAC:SOR	1.4:1	0.216	11.65	50.6	Chick and Ustunol (1998)
	LAC:SOR	1:1	0.216	7.48	156.0	Chick and Ustunol (1998)
	LAC:SOR	1:1	0.104	6.2	156	Chick and Hernandez (2002)
	LAC:SOR[a]	1:1	0.104	1.1	167	Chick and Hernandez (2002)
	LAC:SOR:carnauba wax	3.3:2.3:1	0.104	7.9	31	Chick and Hernandez (2002)
	LAC:SOR:carnauba wax[a]	3.3:2.3:1	0.104	1.9	88	Chick and Hernandez (2002)
	LAC:SOR:candelilla wax	3.3:2.3:1	0.104	8.3	37	Chick and Hernandez (2002)
	LAC:SOR:candelilla wax	3.3:2.3:1	0.104	1.8	74	Chick and Hernandez (2002)
Rennet casein	REN:GLY	0.6:1	0.216	0.83	123.2	Chick and Ustunol (1998)
	REN:GLY	1:1	0.216	2.42	185.4	Chick and Ustunol (1998)
	REN:GLY	1.4:1	0.216	4.5	223.5	Chick and Ustunol (1998)
	REN:SOR	0.6:1	0.216	3.83	4.9	Chick and Ustunol (1998)
	REN:SOR	1:1	0.216	9.53	7.6	Chick and Ustunol (1998)
	REN:SOR	1.4:1	0.216	15.12	17.9	Chick and Ustunol (1998)
Calcium caseinate	CaCAS:GLY	2:1	0.105	4.25	1.45	Banerjee and Chen (1995)
	CaCAS:GLY	2.33:1	0.11	1.6	66.6	Tomasula *et al.* (1998)
	CaCAS:GLY	2.33:1	0.15	1.9	76	Tomasula *et al.* (1998)
	CaCAS:GLY	2.33:1	0.10	7.0	66	Tomasula *et al.* (2003)
	CaCAS:GLY:PPG	2.33:0.75:0.25	0.10	12	88	Tomasula *et al.* (2003)

Table 23.3 *Cont'd*

Dairy protein	Composition	Weight ratio (protein/ plasticizer)	Thickness (mm)	Tensile strength (MPa)	Percent elongation (%)	References
AcCAS	AcCAS:GLY	2.33:1	0.10	4	43	Tomasula *et al.* (2003)
Sodium caseinate	NaCAS	–	0.3364	37.5	2.1	Schou *et al.* (2005)
	NaCAS:GLY	2:1	0.109	13.9	30.8	Banerjee and Chen (1995)
	NaCAS:GLY	2.125:1	0.3577	9.0	13.6	Schou *et al.* (2005)
	NaCAS:GLY	4:1	0.085	17.4–26.7	10.5	Siew *et al.* (1999)
	NaCAS:GLY	2:1	0.085	10.9–11.7	73.7–84.2	Siew *et al.* (1999)
	NaCAS:GLY	4:1	0.10	12.8	24	Khwaldia *et al.* (2004a)
	NaCAS:PEG	4.54:1	0.085	10.9–16.35	5.3	Siew *et al.* (1999)
	NaCAS:PEG	1.9:1	0.085	10.9–13.9	25.4	Siew *et al.* (1999)
	NaCAS:GLY:AMF	10:2.5:1	0.100	11.5	10	Khwaldia *et al.* (2004a)
	NaCAS:GLY:AMF	3.33:0.83:1	0.100	4.8	6	Khwaldia *et al.* (2004a)
CO_2–casein	CO_2–CAS:GLY	2.33:1	0.11	1.2	50.2	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.15	3	74.2	Tomasula *et al.* (1998)
	CO_2–CAS:GLY	2.33:1	0.10	5	56	Tomasula *et al.* (2003)
	CO_2–CAS:GLY:PPG	2.33:0.75:0.25	0.10	7	88	Tomasula *et al.* (2003)
Whey protein isolate	Native WPI:GLY	2.3:1	0.139	3.1	7	Perez-Gago *et al.* (1999)
	WPI:GLY	2.3:1	0.139	6.9	41	Perez-Gago *et al.* (1999)
	WPI:GLY	5.7:1	0.110	29	4	McHugh and Krochta (1994b)
	WPI:GLY	2.3:1	0.110	13.9	30.8	McHugh and Krochta (1994b)
	WPI:SOR	2.3:1	0.110	14	1.6	McHugh and Krochta (1994b)
	WPI:SOR	1:1	0.110	14.7	8.7	McHugh and Krochta (1994b)
	WPI:GLY, 5.5% DH	2.33:1	0.1344	1	40	Sothornvit and Krochta (2000a)
	WPI:GLY, 10% DH	2.33:1	0.1344	2	4	Sothornvit and Krochta (2000a)
Synthetic films	LDPE	–	–	13	500	Salome (1986)
	HDPE	–	–	26	300	Salome (1986)
	Polystyrene	–	–	35–55	1	Houston (1986)
	Plasticized PVC (wrap film)	–	–	15–30	150–350	Audic *et al.* (2003)

[a] Test conditions, 23°C; 75% RH.
NFDM = nonfat dry milk; GLY = glycerol; SOR = sorbitol; PPG = polypropylene glycol; PEG = polyethylene glycol; AMF = anhydrous milk fat; LDPE = low density polyethylene; HDPE = high density polyethylene; PVC = poly(vinyl chloride).

reducing the particle size of granular dry CO_2 casein from 126 μm to 111 μm. The films were still hazy. Reducing the particle size to less than 86 μm resulted in films that were glossy and also transparent. However, reducing particle size decreased the TS of the films and increased WVP.

The gloss and appearance properties of whey protein films are reported in detail in Dangaran and Krochta (2008). Compared to the CO_2 casein films, whey protein films exhibit gloss units near that of shellac (92.9).

23.4 Improvements to edible films

Although the TS and OP of milk-based edible films with added plasticizer approach that of the synthetic films, especially at low %RH, the low %E, the high WVP and in some cases the high solubility of the films in water, precludes their use in most applications. While the amount of added plasticizer tends to improve the %E of the films, it also weakens the TS of the films. Several studies have focused on improving the WVP properties of the films by making various modifications that include the addition of lipids and waxes, or cross-linking agents, or treatment with ultrasound and irradiation.

23.4.1 Addition of lipids or waxes or polysaccharides

Hydrophobic lipids or waxes have been added to milk-based edible films either by creating a stable emulsion with the lipid or forming a bilayer or composite film with the lipid. The hydrophobic lipids or waxes do not allow water to diffuse through them.

Introducing acetylated monoglyceride (AM) or beeswax (BW) to NaCAS in emulsion films resulted in significant improvements in WVP (Avena-Bustillos and Krochta, 1993) with significant reductions compared with the caseinate film. Addition of BW to CaCAS films also resulted in significant reductions in WVP. Increasing concentrations of BW resulted in thicker films but did not improve WVP because the wax was not dispersed effectively. The lipid type and concentration was also found to influence the WVP of the caseinate films. BW, due to its crystalline structure was more effective than stearic acid and AM in reducing WVP of NaCAS films.

Addition of carnauba or candelilla waxes resulted in little improvement of the WVP of lactic acid casein-based emulsion films plasticized with SOR (Chick and Hernandez, 2002). The WVP decreased with increasing wax content but the reduction in WVP was low despite the added wax. The OP of the films with the added wax was comparable to that of films without wax, while TS improved slightly and %E was reduced. Scanning electron microscopy showed that the wax was partially distributed within the protein–water–SOR matrix.

Films made from an emulsion of anhydrous milk fat (AMF), comprising 10–30% of the NaCAS by weight and plasticized with GLY (25%), were

weaker than NaCAS films without AMF, had a decreased % E, and showed no improvement in WVP (Khwaldia *et al.*, 2004a). The loss of mechanical properties of the films was attributed to a loss of continuity of the protein matrix because of the presence of lipid globules. At AMF levels greater than 30%, it was hypothesized that the distribution of lipid globules with high particle size affected the structural cohesion of the polymeric matrix and the emulsion stability, leading to an increase in WVP and a decrease in the film tortuosity, which increased water diffusion. Water does not diffuse through the lipid. OP was found to decrease with increasing amounts of AMF up to 20% and then increased again at AMF levels greater than 20%.

The addition of carrageenans can improve the mechanical properties of CO_2–CAS films plasticized with GLY (20%). CO_2–CAS films were blended in a 1:1 ratio with either lambda (λ-), iota (ι-), or kappa (κ-) carrageenan (Unpublished data, Dangaran and Tomasula, 2007). The carrageenans did not lower WVP, but TS increased from 13.2 MPa to 22.4–23.3 MPa with the addition of κ-carrageenan. The TS of blends containing the carrageenans was approximately the average of the TS of the casein film and that of the carrageenan alone. %E was significantly increased from 5.6% to 42.9% with λ-carrageenan. The improvement in %E appears to be due to the presence of the carrageenan alone which had a %E of 40%. ι- and κ-carrageenan approximately doubled %E. DSC analysis showed that blends of the carrageenans with the protein were miscible as indicated by a single peak on thermograms. The differences in charge densities of the carrageenans compared to the proteins appear to affect their interactions.

Unlike the results for the casein films, the addition of lipids and waxes was found to improve the WVP of whey protein films, but decreased their tensile properties. Films made from an emulsion of WPI and BW, and plasticized with SOR exhibited lower WVP than the WPI films (McHugh and Krochta, 1994a). Decreasing the lipid particle size which increased the number of lipid particles and film tortuousity resulted in a further decrease in WVP and improvement in TS (Perez-Gago and Krochta, 2000). Heat denaturation of the WPI resulted in intermolecular disulfide bonds through thiol-oxidation and thiol-disulfide interchange reactions that further improved the WVP. Reducing the pH of the emulsion below that of the pI of the protein caused WVP to increase due to a sharp change in viscosity with an increase in protein aggregation, which possibly lowered the lipid mobility and reduced the interconnectivity among the lipid droplets.

Lipid concentration and lipid type also influenced WVP of WPI films (McHugh and Krochta, 1994c). Increasing the lipid content of the films resulted in a downward trend in WVP for WPI films with added BW or palmitic acid, but there was a lesser trend with stearyl alcohol. Emulsion films comprised of WPI, lipid, and SOR exhibited a low WVP if the lipid was a fatty acid (palmitic acid, myristic acid) or BW but was twice as high if the lipid was stearyl alcohol, hexadecanol or tetradecanol.

Shellhammer and Krochta (1997) determined the viscoelastic properties of four lipids and waxes, carnauba wax, candelia wax, milkfat fraction and BW, and showed that the viscoelastic milkfat and BW improved WVP in whey films more than carnauba or candelia waxes. The milkfat and BW deform to a greater extent during drying to form an intact lipid network due to their more plastic nature.

In order to enhance or reduce lipid deformability and the ability to yield an internal interconnecting lipid network in the films, Perez-Gago and Krochta (2000) dried WPI films in an emulsion with either BW, anhydrous milkfat fraction, or candelilla wax. Significantly lower WVP was observed for films dried at 80°C and 40% RH compared to films dried at 25 or 40°C and 40% RH. The mechanical properties of the films were not modified by the drying temperature.

23.4.2 Cross-linking of milk protein films

For synthetic polymers, cross-linking is used to decrease polymer chain mobility to increase resistance to vapor and gas transport (Kumins, 1965), by joining two or more functional groups of the polymer by a covalent bond. pH adjustment, ionic cross-linking, through the introduction of calcium ion, and cross-linking using heating, γ-irradiation or food grade enzymes have been explored to improve the mechanical and barrier properties of edible films.

pH treatment

The WVP of NaCAS films, soaked for 1 minute in sodium acetate, sodium ascorbate or calcium ascorbate buffers, and adjusted to pH 4.6 or soaked for 1 minute in calcium chloride solution and then adjusted to pH 9.6, was reduced by approximately 40% (Avena-Bustillos and Krochta, 1993). The WVP was lowered possibly due to increased protein–protein interaction, as indicated by the decreased film thickness of the treated films.

γ-irradiation

Treatment of solutions of NaCAS and CaCAS, with or without added GLY, by γ-irradiation formed edible films with improved mechanical properties, such as puncture strength and puncture deformation (Brault *et al.*, 1997). γ-irradiation results in the formation of bityrosine, a covalently bound biphenol, produced by the reaction of two tyrosyl radicals or a tyrosyl radical plus a tyrosine molecule. GLY increased the formation of cross-links within protein chains and was found to improve the mechanical strength and film flexibility. The greatest effects of irradiation dose on the mechanical properties of the films occurred at GLY:caseinate ratios of 1:2 and 2:3, leading to branching of polypeptide chains to form a 3-D network. The addition of $CaCl_2$ to CaCAS solution was found to facilitate a reduction

in the salt bridges and electrostatic bonds facilitating the formation of bityrosine during irradiation. Gels were formed at irradiation doses greater than 16 kGy (Ressouany *et al.*, 1998).

Vachon *et al.* (2000) combined thermal treatment and γ-irradiation and showed that the molecular weight of CaCAS increased, the mechanical strength of the films increased and solubility of the films in water decreased. WPI did not show similar behavior because cross-links were not generated due to the low tyrosine content of the protein. However, WPI could be substituted for CaCAS in films, resulting in an increase of the puncture strength of the treated films. γ-irradiation may induce more intermolecular interactions and the formation of inter- and/or intra-molecular covalent cross-links in the film-forming solutions (Ouattara *et al.*, 2002). A reduction in WVP from 2.07 (unirradiated) to 1.38 g mm/m^2 d/mm Hg (irradiated) was noted for CaCAS-WPI films in the ratio of 25:75.

Transglutaminase

Transglutaminase (TGase) is a food-grade enzyme that uses the acyltransferase mechanism, in the presence of Ca ion, to catalyze the transfer of gamma-carboxyamide (acyl donor) of a peptide-bound glutamine residue to the gamma-amine (acyl acceptor) of lysine residues along protein chains (Mahmoud and Savello, 1992). Intramolecular and intermolecular ε-(γ-glutaminyl)lysine cross-links are generated when the ε-primary amine of lysine is bound to a glutamine-containing protein. It was shown to effectively cross-link whey-based formulations containing a modified whey powder (35% protein) (Aboumahmoud and Savello, 1990) and decrease the solubility of 1:1 mixtures of α-LA and β-LG with added GLY in buffers at various pH and temperature (Mahmoud and Savello, 1992). The films were protease-digestible (Mahmoud and Savello, 1993).

The addition of TGase to WPI films, plasticized with GLY (Yildirim and Hettiarachchy, 1998), did not improve WVP compared to the control, possibly due to the creation of additional, or an increase in size of, existing pores. TS increased from 5.64 MPa for the untreated whey protein film to 12.53 MPa.

The globular nature of the whey proteins, especially β-LG, prevents cross-linking by TGase, except if denatured. Cross-linking is further limited by the formation of non-covalent aggregates between calcium and β-LG, induced by electrostatic interaction (Faergemand *et al.*, 1998). Sharma *et al.* (2001) determined the susceptibility to cross-linking by TGase of the individual milk proteins in skim milk. In both heated and unheated milk, there was a reduction in the monomeric forms of κ- and β-caseins, indicating that these proteins were most susceptible to TGase-induced cross-linking. Only preheated β-LG was susceptible to the same amount of cross-linking as α-LA, preheated or not. Nieuwenhuizen *et al.* (2004) demonstrated the modification of the lysine and glutamine residues in native β-LG to improve the accessibility of the residues to reaction with TGase under nonreducing

and nondenaturing conditions, with a possible application for the creation of novel foods.

In another study, Sharma *et al.* (2002) showed that commercial α-LA was susceptible to more cross-linking by TGase than that from the study by Aboumahmoud and Savello (1990). Reduction of disulfide bonds in the commercial protein was found to be unnecessary because structural modifications of the protein occurred during manufacture exposing available sites for TGase reaction.

Chambi and Grosso (2006) cross-linked NaCAS, gelatin, and mixtures of the two, using TGase to produce edible films that showed improvement in the mechanical and barrier properties of the films, depending on the ratio of casein to gelatin in the film. The films contained 25 g GLY/100 g protein. TS of the NaCAS (~12 MPa), gelatin film(~36 MPa) and of the caseinate–gelatin films, which increased with increasing gelatin content, were not significantly different from that of the untreated films. On the other hand, % E of the untreated films increased from 9% for NaCAS film to a maximum value (~27%) at caseinate:gelatin ratios of 75:25 and 50:50, and then decreased to ~9% for the gelatin film. With TGase treatment, %E of the NaCAS film approximately doubled while that of the gelatin film was not significantly different. The greatest increase in %E was noted for blends containing 75:25 caseinate:gelatin, showing % E of 56.59% compared to a value of 27.2% for the untreated film. This film also showed an anomalous decrease in WVP with TGase treatment, while the others showed increases in WVP. It was concluded that cross-linking may have increased the proportion of hydrophobic segments on the surface of the film, which are mostly provided from the NaCAS, thus leading to a lower WVP.

O'Connell and de Kruif (2003) showed that TGase treatment at either 0 or 35°C affects the association behavior of β-casein. TG appeared to freeze the micellization process, indicating that the associative state of β-casein should be considered prior to treatment with the enzyme. The changes appeared to be due to charge modification. This finding may be of interest in the design of future experiments involving edible films.

Building on the observations that ultra-high temperature (UHT) treatment of milk seemed to produce a more open, looser casein micelle structure, Bönisch *et al.* (2004) heated NaCAS solutions to 140°C and immediately cooled them using ice water. The UHT-treated NaCAS solutions and controls were then treated with TGase. TGase treatment of NaCAS resulted in a degree of protein polymerization of about 40% after 90 min compared to 60% for TGase treatment of NaCAS that was heated at 140°C for 20 s. This finding may have application in the development of stronger films with improved WVP.

Tyrosinase

While the caseins are more susceptible to cross-linking using TGase than the whey proteins, mushroom tyrosinase (E.C.1.14.18.1), a polyphenol

oxidase, has been shown to be an effective cross-linking agent for the whey proteins, α-LA and β-LG (Thalman and Lotzbeyer, 2002), but its ability to improve the mechanical and barrier properties of dairy-based films has not yet been reported. Tyrosinase first induces the formation of *o*-quinones in the presence of low molecular weight (MW) phenolic compounds or from the tyrosine residues. The low MW compounds, such as caffeic or chlorogenic acid, act as a substrate for the cross-linking process and are oxidized to the *o*-quinone structure. The *o*-quinones may then interact with each other or react with amino and sulphydryl groups or pyrrolidine side chains of the proteins (Thalman and Lotzbeyer, 2002; Seo *et al.*, 2003; Lantto *et al.*, 2005). α-LA and β-LG polymers with a MW greater than 300 kDa were produced from the proteins with initial MW of 14 kDa and 18 kDa, respectively, after treatment with tyrosinase (Thalman and Lotzbeyer, 2002). For α-LA, addition of caffeic acid was not necessary but increasing temperatures up to 50°C alone resulted in polymers with molecular weight greater than 300. The polymerization of β-LG required use of an external phenolic source (Thalman and Lotzbeyer, 2002). Onwulata and Tomasula (2008) used tyrosinase to investigate the gelling properties of microparticulated WPI and CaCAS slurries in the presence of alginic acid. Stable gels were obtained with increasing amounts of tyrosinase. While the gels were designed as replacements for carbohydrates in low-fat foods, the slurries have potential as films.

Genipin

Genipin, obtained from the iridoid glucoside, geniposide, present in the fruit of *Gardenia jasminoides* Ellis, is another natural cross-linking agent which has potential use in improving the mechanical and barrier properties of casein and whey protein films. However, it characteristically gives a deep blue color to primary amino acids, such as the lysine residues of proteins. Bigi *et al.* (2002) showed that the extensibility, the swelling properties, and the enthalpy of denaturation, of gelatin films cross-linked with genipin were similar to that using glutaraldehyde but showed a greater thermal stability. The mechanical properties and stability of chitosan and chitosan blend films in water were also improved when cross-linked with genipin, suggesting potential applications in biomaterials (Jin *et al.*, 2004). Under basic conditions, genipin undergoes a ring-opening polymerization prior to cross-linking with chitosan. The crosslinked bridge consists of polymerized genipin macromers or oligomers (7–88 monomer units) (Mi *et al.*, 2005). Under acidic or neutral conditions, genipin reacts with the primary amino groups on chitosan to form heterocyclic amines. The heterocyclic amines are further associated to form cross-linked networks with short chains of dimer, trimer, and tetramer bridges.

Preliminary work in our laboratory to investigate the use of genipin for improving the properties of CO_2–CAS, CaCAS, and whey protein films, shows that reaction of the proteins with genipin is favored under neutral

and basic conditions, the solubility of the films in water is decreased and TS increases.

Other treatments

Banerjee *et al.* (1996) demonstrated that ultrasound treatment of NaCAS and WPC solutions with a 1:2 ratio of GLY:protein improved the TS of the films compared to controls. However, WVP and %E were not affected. Ultrasound may have created greater molecular order in the films, enabling greater inter-molecular attraction, and therefore enhanced TS. WPC films showed smaller particle and lipid droplet sizes, which may also have enabled greater molecular attraction.

23.5 Milk proteins in composite films

Paper is the most commonly used packaging material and it is coated with wax or laminated to synthetic films or aluminum foil to improve its resistance to moisture, oil or grease, and to give oxygen barrier properties. Researchers have tested the properties of edible milk protein-based films in composite structures with the intention of placing the edible film side of the composite in contact with the food and to facilitate composting or recycling.

WPI solutions containing GLY spread on pulp paper improved the printability of the paper (Han and Krochta, 1999). WVP of the paper was also improved because WPI impregnated the porous paper structure, but this also increased the maximum amount of water absorbed by the paper. However, the WPI did not significantly change the mechanical and optical properties of the paper (Han and Krochta, 2001) and paper coated with 18 g/m^2 of WPI demonstrated increased oil resistance. Both denatured and native WPI showed grease barrier properties similar to that of commercial polyvinyl alcohol and fluorocarbon coatings and were highly impermeable to grease penetration after four hours when coated on paperboard (Chan and Krochta, 2001). Lin and Krochta (2003) found similar results for WPC-80 coatings on paperboard. Using sucrose as a plasticizer, the grease resistance properties of WPC-80 were retained after ambient storage and during extended testing times of 16 hours.

The oxygen barrier properties of WPI with various plasticizers have been exploited to create a laminated structure with polypropylene (PP). An important consideration in designing a laminate structure is the surface energy of the synthetic polymer and surface tensions of the liquids and their contact angle when coating synthetic substrates with the protein formulations (Hong *et al.*, 2004). Non-polar PP was first treated with corona discharge to improve its adherence to the WPI coating. OP of the laminated PP films was reduced 4-fold at 40°C with the addition of a WPI:GLY coating at 50% RH. OP of the laminated films was sensitive to RH and

increased significantly with RH in the range from 30–85% range, approaching that of the uncoated PP film at 80% RH (Hong and Krochta, 2003). In another study (Hong and Krochta, 2006), OP was significantly reduced using either WPI or WPC coating at 40°C and 50% RH on both PE and PP laminates. OP of the PP laminates were significantly lower than that of the PE laminates due to differences in the intrinsic characteristics of the base films. Excellent OP properties were noted for both laminates at low to intermediate RH. WPI, plasticized with GLY, PEG, SOR or sucrose, and coated onto PP films resulted in films with TS of up to 70 MPa and %E as high as 135%.

The mechanical and WVP properties of edible films containing a whey powder and NaCAS mixture, with whey powder as the principal component, were improved when laminated to corn-zein based films (Cho *et al.*, 2002); however, %E was significantly reduced. Whey powder, containing 0.11 g protein/g powder is significantly less expensive than WPC or WPI. Addition of stearic acid to the corn-zein layer resulted in a reduced WVP compared to the whey-caseinate film.

A study conducted to determine the mechanical and WVP properties of blends or bilayers of NaCAS–pullulan films plasticized with SOR (Kristo and Biliaderis, 2006; Kristo *et al.*, 2007) showed that varying the ratio of the polymers changed the mechanical properties of the film but not WVP. Increasing the pullulan content decreased TS and increased %E, showing that the pullulan imparts flexibility and NaCAS stiffness to the films. Application of BW improved the WVP of the films.

23.6 Modifying the properties of edible films through processing

Most of the studies to determine the properties of edible milk protein films and coatings have been conducted on films prepared through solvent casting on the laboratory-scale. Larger scale production is needed to produce samples for applications studies.

There are several examples in the patent literature for the production of casein films using extrusion methods. However, little information is provided on the mechanical and barrier properties of these films. Kozempel and Tomasula (2004) developed a continuous solvent casting process to make casein films from CaCAS or CO_2–CAS. Hot air drying was used in the process. The mechanical properties of the films were similar to those made on the laboratory-scale. Nonfat dry milk was substituted for up to 20% of the CaCAS or CO_2–CAS content with no loss in physical properties.

Casein films have also been made through the wet-spinning process. In this method, acid casein is extruded with water and sodium hydroxide to form sodium caseinate, discharged into a coagulating bath, and then

collected on a roller (Frinault *et al.*, 1997). Because of the chemicals used in the bath to harden the casein films, the films were rendered inedible. However, the films had low solubility in water, WVP was approximately 1.40 g-mm/m^2 hkPa, TS was 4.5 MPa and %E was 68.6%, greater than that reported for solvent cast films, which may be due to shearing effects of extrusion on the molecular structure of casein.

Müller-Buschbaum *et al.* (2006) prepared casein films using a spin-coating technique, a method that is used to prepare thin and ultrathin films from synthetic polymers, on base-treated glass surfaces. The glass surfaces were treated to assure wetability of the casein solution. Films dried in less than 30 s and the moderate pressures introduced by spin-coating force the micelles to rearrange into a more compact structure. This method is still in the experimental stages but has potential for the preparation of advanced coatings of casein in laminate structures or on foods.

Sheets made by compression molding of WPI:GLY mixtures were compared to solvent cast films having the same concentration of WPI and GLY (Sothornvit *et al.*, 2003, 2007). Operating temperature and pressure of the process did not affect the mechanical properties of the films but increasing amounts of GLY resulted in films with decreased TS and no change in %E. Compression molded films had higher WVP than solvent cast films because the compression molded films demonstrated less protein cross-linking, were thicker and were more soluble in water.

Dangaran and Krochta (2008) report details of extrusion studies which demonstrate that extrusion operating conditions provide sufficient heat-denaturing and cross-linking to produce transparent, flexible whey protein sheets with improved tensile properties relative to cast films. Hernandez-Izquierdo *et al.* (2008) showed that extruded WPI plasticized with GLY had tensile properties comparable to solvent cast and compression molded films but exhibited greater %E. A WPI film containing 50% GLY had a %E of 132% compared with 68% for a solvent cast WPI film containing 55% GLY. It was concluded that extrusion created a greater alignment of the polymer molecules in the machine direction as the sheets exited the extruder. Onwulata *et al.* (2003, 2006) and Tunick and Onwulata (2006) have demonstrated that shearing of the protein from the extrusion process leads to creation of aligned whey protein polymers.

23.7 Potential Applications

The film-forming properties of casein have long been exploited in non-food applications (Audic *et al.*, 2003). While research on investigation of the mechanical and barrier properties of casein and whey proteins is active, research lags on applications of casein and whey films in food packaging and preservation, to improve the appearance and quality of foods, and as a barrier layer in packaging systems.

Some of the potential applications of casein and whey films and coatings as moisture and gas barriers for fresh fruits and vegetables, meats, cereals, nuts and frozen foods are reported by Khwaldia *et al.* (2004) and Vargas *et al.* (2008). A more recent review on applications of whey protein based films and coatings includes nuts and peanuts, eggs, confectionary products, meat product, fruits and vegetables (Dangaran and Krochta, 2008). Yildirim and Hettiarachchy (1998) suggested that TGase-cross-linked WPI films could be used as a wrapping to prevent quality changes in products such as meat pies and high-moisture cakes requiring films permeable to water vapor. However, the addition of milk-based films and coatings to fresh fruits and vegetables would require additional labeling on the package to alert consumers with milk allergies, intolerances, religious-based dietary restrictions, or who maintain a vegetarian lifestyle (Gennadios, 2004). Limiting applications to dairy-based applications, such as films and coatings for cheese, or fresh, baked and frozen products expected to be consumed with or to contain milk-based proteins is a more logical approach and applications research should focus on these types of products (Albert and Mittal, 2002; Guillard *et al.*, 2003; Schou *et al.*, 2005). Casein films have also been explored as tablet coatings (Abu Diak *et al.*, 2007).

Recent research efforts have focused on the development of active whey protein films for packaging applications. Active packaging interacts directly with a food or headspace of the product (Ozdemir and Floros, 2004). Whey-based films alone have the advantage of being able to be in direct contact with food and in active packaging systems could contain additional flavors, natural oxygen scavengers and antimicrobials (Lee *et al.*, 2008; Dangaran and Krochta, 2008). Cagri *et al.* (2004) have reviewed the various types of protein-based and lipid-based edible films and a wide range of antimicrobial agents that could be used or are currently used to enhance the safety and shelf-life of ready-to-eat foods.

Huppertz and de Kruif (2008) demonstrated the creation of stable nanogel particles by cross-linking all caseins within the casein micelle using TGase from which all micellar calcium phosphate can be removed. The particles may have applications in the encapsulation of minerals, vitamins and pharmaceuticals, as well as to add flavors, additives and other components to foods.

23.8 Future trends

Current milk-protein based research efforts on edible films arose from the need to utilize surplus dairy proteins and was directed to improving the mechanical and barrier properties of films made with these proteins. This research needs to continue, especially for applications research, but more attention needs to be directed to structuring the films through consideration of the conformation of the protein in the film-forming solution as affected by

environmental variables (Lent *et al.*, 1998; Anker *et al.*, 2000; Farrell *et al.*, 2003a, 2003b; O'Connell and de Kruif, 2003; Lefèvre *et al.*, 2005; Lencki, 2005; Qi, 2007). Just as importantly, future research needs to extend to the production of the films on a large-scale basis with pilot-plant scale equipment used by the food industry to produce large quantities of the films for food applications and shelf-life studies. Little is known about the endurance of the films during refrigeration, freezing, baking and microwaving. Cast films may have immediate application as reinforcements of foil packaging and for food protection; e.g. to reinforce the foil lids of dairy food containers while protecting the contents, or to protect entrees in microwaveable meals. Little is known about the heat-sealing properties of the films.

The impact of processing on the mechanical and barrier properties of the films also needs to be established. Some work has been done to date using a continuous process, compression molding, injection molding and extrusion. These techniques have been shown to improve the mechanical properties of the films. However, the proteins are heat sensitive which can cause color and sensory changes; this needs to be addressed. Wet spinning is another area that deserves more attention, possibly investigating the use of tyrosinase, TGase, genipin and other natural cross-linkers as replacements for the chemicals typically used in the process.

Coatings of foods with milk-based coatings on a commercial basis may be easier to achieve than film production at the present time. Techniques used by the food industry to coat foods, such as panning, fluidized bed coating, spray coating, and dipping, require little modification for use with edible milk-based coating solutions. However, research is still needed to establish operation parameters and processing protocols for each food product.

The use of milk-based films as part of multilayered packaging systems has not yet begun in earnest. However, this may prove to be the best outlet for edible films and coatings. Research has demonstrated that application of milk-based protein films to synthetic packaging improves the oxygen barrier properties of the film significantly. Milk-based films have the potential to reduce the amount of synthetic films or aluminum foil used, and facilitate biodegradability (Li and Chen, 2000; de Vlieger, 2003) However, research is needed to determine compatible film systems, the stability of the protein film layer in contact with the food, and resistance to microbial degradation as a function of shelf-life, for example. While costs are part of the drivers for selection of a packaging system, the costs of synthetic films are rising. Kozempel *et al.* (2003) have estimated that the hot-air drying costs for a 0.23 mm milk-protein film are \$0.26/m^2, assuming a 10% initial solids concentration in the film-forming solution. In addition, industry has incorporated sustainability of their operations as part of their business planning, and is committed to reducing packaging.

Research is also scarce on the creation of new blended films made from synthetic film material, or the biodegradable polylactic acid (PLA), with

milk-based proteins and other materials. The blends would reduce the use of the synthetic feedstock. Prototype cups (not films) made in our laboratory contained up to 25% WPI as a replacement for some of the PLA and PE material. Information on the properties of synthetic films that should be targeted in blends is found in texts such as Massey (2003).

Research on the use of nanoparticles to strengthen edible milk protein films for multi-layer packaging options is currently underway in our laboratory and is an unexplored area. Inclusion of nanoparticles such as montmorillonite clay or calcium phosphate may improve the handling of the films for manufacturing. Inclusion of whey (Bonnaillie and Tomasula, 2008) or casein fractions, pending commercial availability, may have similar impact by imparting their individual functionality to film properties.

23.9 Sources of further information and advice

The use of films or coatings made from proteins and other biobased materials has been the subject of many review articles and book chapters (Kester and Fennema, 1986; Guilbert, 1986; Chen, 1995; Krochta and De Mulder-Johnson, 1997; Debeaufort *et al.*, 1998; Korhonen, 2002; Tharanathan, 2003; Gennadios, 2004; Dangaran and Krochta, 2008).

General information on edible films, coatings, and applications in commercial use may be found at several vendor websites. Examples include: Watson, Inc., a manufacturer of edible films http://www.watson-inc.com/film_edible.php.; Ascona Ingredients Ltd, http://www.ediblefilms.com; a manufacturer of edible films, and Mantrose-Haeuser, http://www.mbzgroup.com, another manufacturer of edible films and coatings. Several use USDA/ARS technology and that developed by other research institutions in the field. Another website providing information on edible films is that sponsored by the California Dairy Research Board, http://www.ediblefilms.org. Information on commercial packaging use is found at industry websites such as http://www.packworld.com and http://www.innoviafilms.com. General information on new commercial developments in the dairy foods area may be found at http://www.dairyfoods.com. The above list is not meant to be exhaustive.

New research on milk protein edible films and other edible films is published in The *Journal of Food and Agricultural Food Chemistry*, the *Journal of Food Science*, and the *Journal of Dairy Science*. Use of search engines and software for publishing and managing bibliographies will yield most, if not all, research conducted to date on milk protein edible films not included in this chapter, as well as patents and information on films for non-food use. Research is also presented at annual meetings of the American Chemical Society (ACS), the American Institute of Chemical Engineers (AICHE), the Institute of Food Technologists (IFT), the American Dairy Science Association (ADSA), as well as at International meetings such as that held by the International Dairy Federation (IDF).

23.10 References

ABOUMAHMOUD, R and SAVELLO, P (1990), 'Crosslinking of whey protein by transglutaminase', *J Dairy Sci* 73:256–263.

ABU DIAK, O, BANI-JABER, A, AMRO, B, JONES, D and ANDREWS, G P (2007), 'The manufacture and characterization of casein films as novel tablet coatings', *Trans IChemE, Part C, Food and Bioproducts Processing*, 85, C3, 284–290.

ALBERT, S and MITTAL, G S (2002), 'Comparative evaluation of edible coatings to reduce fat uptake in a deep-fried cereal product', *Food Research International*, 35, 445–458.

ALCANTARA, C R, RUMSEY, T R and KROCHTA, J M (1998), 'Drying rate effect on the properties of whey protein films', *J Food Process Engineering*, 21, 387–405.

ANKER, M, STADING, M and HERMANSSON, A-M (2000), 'Relationship between the microstructure and the mechanical and barrier properties of whey protein films', *J Agric Food Chem*, 48, 3806–3816.

ASTM (1989) Standard D523-89, *Standard test methods for specular gloss D523-89*, ASTM International, West Conshohocken, PA.

ASTM (1995) Standard E96-95, *Standard test methods for water vapor transmission of materials*, ASTM International, West Conshohocken, PA.

ASTM (1995a) Standard D3985-95, *Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor*, ASTM International, West Conshohocken, PA.

ASTM (1999) Standard D4039-93, *Standard test method for reflection haze of high-gloss surfaces*, ASTM International, West Conshohocken, PA.

ASTM (2001) Standard D882-01, *Standard test method for tensile properties of thin plastic sheeting*, ASTM International, West Conshohocken, PA.

AUDIC, J-L, CHAUFER, B and DAUFIN, G (2003), 'Non-food applications of milk components and dairy co-products: A review', *Lait*, 83, 417–438.

AVENA-BUSTILLOS, R J and KROCHTA, J M (1993), 'Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content'. *J Food Sci*, 58(4), 904–907.

BANERJEE, R and CHEN, H (1995), 'Functional properties of edible films using whey protein concentrate', *J Dairy Sci*, 78, 1673–1683.

BANERJEE, R, CHEN, H and WU, J (1996), Milk protein-based edible film mechanical strength changes due to ultrasound process, *J Food Sci*, 61(4), 824–828.

BANKER, G S (1966), 'Film coating theory and practice', *J Pharm Sci* 55(1), 81–89.

BIGI, A, COJAZZI, G, PANZAVOLTA, S, ROVERI, N, and RUBINI, K (2002), 'Stabilization of gelatin films by crosslinking with genipin', *Biomaterials*, 23, 4827–4832.

BÖNISCH, M P, LAUBER, S and KULOZIK, U (2004), 'Effect of ultra-high temperature treatment on the enzymatic cross-linking of micellar casein and sodium caseinate by transglutaminase', *J Food Sci*, 69, E398–E404.

BONNAILLIE, L M and TOMASULA, P M (2008), Whey processing and fractionation, in Onwulata C and Huth P, *Whey Processing, Functionality and Health Benefits*, Institute of Food Technologists Series, Wiley-Blackwell, 15–38.

BRAULT, D, D'APRANO, G and LACROIX, M (1997), 'Formation of free-standing sterilized edible-films from irradiated caseinates', *J Agric Food Chem*, 45, 2964–2969.

BUONOCORE, G G, DEL NOBILE, M A, DI MARTINO, C, GAMBACORTA, G, LA NOTTE, E and NICOLAIS, L (2003), 'Modeling the water transport properties of casein-based edible coating', *J Food Engineering*, 60, 99–106.

CAGRI, A, USTUNOL, Z and RYSER, E T (2004), 'Antimicrobial edible films and coatings', *J Food Protection*, 67, 833–848.

CHAMBI, H and GROSSO, C (2006), 'Edible films produced with gelatin and casein cross-linked with transglutaminase', *Food Research International*, 39, 458–466.

CHAN, M A and KROCHTA, J M (2001), 'Grease and oxygen barrier properties of whey protein isolate-coated paperboard', *TAPPI*, 84, 57.

CHEN, H (1995), 'Functional properties and applications of edible films made of milk proteins', *J Dairy Sci*, 78, 2563–2583.

CHICK, J and USTUNOL, Z (1998), 'Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films', *J Food Sci*, 63, 1024–1027.

CHICK, J and HERNANDEZ, R J (2002), 'Physical, thermal, and barrier characterization of casein–wax-based edible films', *J Food Sci*, 67, 1073–1075.

CHO, S Y, PARK, J-W and RHEE, C (2002), 'Properties of laminated films from whey powder and sodium caseinate mixtures and zein layers', *Lebensm.-Wiss. U-Technol*, 35, 135–139.

CHURAEV, N V (2003), 'Surface forces in wetting films', *Advances in Colloid and Interface Science*, 103, 197–218.

COUPLAND, J N, SHAW, N B, MONAHAN, F J, O'RIORDAN, E D and O'SULLIVAN, M (2000), 'Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films', *J Food Engineering*, 43, 25–30.

DANGARAN, K L, COOKE, P and TOMASULA, P M (2006), 'The effect of protein particle size reduction on the physical properties of CO_2-precipitated casein films', *J Food Sci*, 71, E196–201.

DANGARAN, K L and KROCHTA, J M (2007), 'Preventing the loss of tensile, barrier and appearance properties caused by plasticiser crystallisation in whey protein films', *International Journal of Food Science and Technology*, 42, 1094–1100.

DANGARAN, K and KROCHTA, J M (2008), Whey protein films and coatings, in Onwulata C and Huth P, *Whey Processing, Functionality and Health Benefits*, Institute of Food Technologists Series, Wiley-Blackwell, 133–168.

DEBEAUFORT, F, QUEZADA-GALLO, J-A and VOILLEY, A (1998), Edible films and coatings: Tomorrow's packagings: A review. *Critical Reviews in Food Sci*, 38, 299–313.

DE VLIEGER, J J (2003) 'Green plastics for food packaging'. In Ahvenainen, R, *Novel Food Packaging Technologies*, Cambridge, Woodhead, 519–534.

FAERGEMAND, M, OTTE, J and QVIST, K B (1998), 'Crosslinking of whey proteins by enzymatic oxidation', *J. Agricultural and Food Chemistry*, 46, 1326–1333.

FARRELL, H M JR, BROWN, E M, MALIN, E L and HOAGLAND, P D (2003a), Higher order structures in casein: A paradox. In Fox, P F and McSweeny, P H, *Advanced Dairy Chemistry: Proteins, 3rd ed*, New York, Plenum-Kluwar Inc, 96–116.

FARRELL, H M, COOKE, P H, WICKHAM, E D, PIOTROWSKI, E G and HOAGLAND, P D (2003b), 'Environmental influences on bovine κ-casein: Reduction and conversion to fibrillar (amyloid) structures', *J Protein Chem*, 22, 259–273.

FRINAULT, A, GALLANT D J, BOUCHET, B and DUMONT, J P (1997), 'Preparation of casein films by a modified wet spinning process', *J. Food Sci*, 62, 744–747.

GENNADIOS, A (2004), 'Edible films and coatings from proteins'. In Yada R Y, *Proteins in Food Processing*, Cambridge, Woodhead, 442–467.

GUILBERT, S (1986), 'Technology and application of edible protective films'. In Mathiouthi M, *Food Packaging and Preservation*, London, Elsevier Applied Science Publishers, 371–394.

GUILLARD, V, BROYART, B, BONAZZI, C, GUILBERT, S and GONTARD, N (2003), 'Preventing moisture transfer in a composite food using edible films: Experimental and mathematical study', *J Food Science*, 68, 2267–2277.

HAN, J H and KROCHTA, J M (1999), 'Wetting properties and water vapor permeability of whey-coated paper', *Transactions of the ASAE*, 42, 1375–1382.

HAN, J H and KROCHTA, J M. (2001) 'Physical properties and oil absorption of whey protein coated paper', *J Food Science*, 66, 294–299.

HERNANDEZ-IZQUIERDO, V M, REID, D S., MCHUGH, T H., BERRIOS, DE J and KROCHTA, J M (2008), 'Thermal transitions and extrusion of glycerol-plasticized whey protein mixtures', *J Food Science* 73, E169–E175.

HONG, S-I and KROCHTA, J M (2003), 'Oxygen barrier properties of whey protein isolate coatings on polypropylene films', *J Food Science*, 68, 224–228.

HONG, S-I, HAN, J H and KROCHTA, J M (2004), 'Optical and surface properties of whey protein isolate coatings on plastic films as influenced by substrate, protein concentration, and plasticizer type', *J Applied Polymer Sci*, 92, 335–343.

HONG, S-I and KROCHTA, J M (2006), 'Oxygen barrier performance of whey protein-coated plastic films as affected by temperature, relative humidity, base film and protein type', *J Food Engineering*, 77, 739–745.

HOUSTON, J S (1986), 'Polystyrene'. In: *The Wiley Encyclopedia of Packaging Technology*; Bakker, M, John Wiley & Sons., New York.

HUPPERTZ, T and DE KRUIF, C G (2008), 'Structure and stability of nanogel particles prepared by internal cross-linking of casein micelles', *International Dairy Journal*, 18, 556–565.

JIN, J, SONG, M and HOURSTON, D J (2004), 'Novel chitosan-based films cross-linked by genipin with improved physical properties', *Biomacromolecules*, 5, 162–168.

JORDAN, P J, LAY, K, NGAN, N and RODLEY, G F (1987), 'Casein precipitation using high pressure carbon dioxide', *N Z J Dairy Sci Technol*, 22, 247–256.

KAYA, S and KAYA, A (2000), 'Microwave drying effects on properties of whey protein isolate edible films', *J Food Engineering*, 43, 91–96.

KESTER, J J and FENNEMA, O R (1986), 'Edible films and coatings: A review', *Food Technol*, 40, 47–59.

KHWALDIA, K, PEREZ, C, BANON, S, DESOBRY, S and HARDY, J (2004) 'Milk proteins for edible films and coatings', *Critical Reviews in Food Science and Nutrition*, 44, 239–251.

KHWALDIA, K, BANON, S, DESOBRY, S and HARDY, J (2004a), 'Mechanical and barrier properties of sodium caseinate–anhydrous milk fat edible films', *Int. J. Food Sci. Tech*, 39, 403–411.

KILARA, A and VAGHELA, M N, (2004), Whey proteins. In Yada R Y, *Proteins in Food Processing*, Cambridge, Woodhead, 72–99.

KORHONEN, H (2002), 'Technology options for new nutritional concepts', *Int. J. Dairy Tech*, 55, 79–88.

KOZEMPEL, M, TOMASULA, P M and MCALOON, A (2003), 'Drying kinetics of calcium caseinate', *J Agric Food Chem*, 51, 773–776.

KOZEMPEL, M and TOMASULA, P M (2004), 'Development of a continuous process to make casein films', *J Agric Food Chem*, 52, 1190–1195.

KRISTO, E and BILIADERIS, C G (2006), 'Water sorption and thermo-mechanical properties of water/sorbitol-plasticized composite biopolymer films: Caseinate–pullulan bilayers and blends', *Food Hydrocolloids*, 20, 1057–1071.

KRISTO, E, BILIADERIS, C G and ZAMPRAKA, A (2007), 'Water vapour barrier and tensile properties of composite-pullulan films: Biopolymer composition effects and impact of beeswax lamination', *Food Chemistry*, 101, 753–764.

KROCHTA, J M and DE MULDER-JOHNSON, C (1997), 'Edible and biodegradable polymer films', *Food. Technol*, 51, 61–74.

KUMINS, C A (1965), 'Transport through polymer films', *J Polymer Sci*, Part C, 10, 1–9.

LANTTO, R, HEINE, E, FREDDI, G, LAPPALAINEN, A, MIETTINEN-OINONEN, A, NIKU-PAAVOLA, M-L and BUCHERT, J (2005), 'Enzymatic modification of wool with tyrosinase and peroxidase', *J Textile Institute*, 96, (2), 109–116.

LEE, J-W, SON, S-M and HONG, S-I (2008), 'Characterization of protein-coated polypropylene films as a novel composite structure for active food packaging application', *J Food Engineering*, 86, 484–493.

LEFÈVRE, T, SUBIRADE, M and PÈZOLET, M (2005), 'Molecular description of the formation and structure of plasticized globular protein films', *Biomacromolecules* 6, 3209–3219.

LENCKI, R W (2007), 'Evidence for fibril-like structure in bovine casein micelles', *J Dairy Sci* 90, 75–89.

LENT, L E, VANASUPA, L S and TONG, P S (1998), 'Whey protein edible film structures determined by atomic force microscope', *J Food Science*, 63, 1–4.

LI, J and CHEN, H (2000), 'Biodegradation of whey protein-based edible films', *J Polymers and the Environment*', 8, 135–143.

LI-CHAN, E C Y (2004), Properties of proteins in food systems: An introduction. In Yada R Y, *Proteins in Food Processing*, Cambridge, Woodhead, 2–28.

LIN, S-Y and KROCHTA, J M (2003), 'Plasticizer effect on grease barrier and color properties of whey-protein coatings on paperboard', *J Food Science*, 68, 229–233.

LONGARES, A, MONAHAN, F J, O'RIORDAN, E D and O'SULLIVAN, M (2004), 'Physical properties and sensory evaluation of WPI films of varying thickness', *Lebensm.-L Wiss. U-Technol*, 37, 545–550.

MAHMOUD, R and SAVELLO, P A (1992), 'Mechanical properties of and water vapor transferability through whey protein films', *J Dairy Sci*, 75, 942–946.

MAHMOUD, R and SAVELLO, P A (1993), 'Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein', *J Dairy Sci*, 76, 29–35.

MASSEY, L.K. 2003. *Permeability properties of plastics and elastomers. A guide to packaging and barrier materials*. Plastics Design Library. Norwich, NY.

MAUER, L J, SMITH, D E and LABUZA, T P (2000), 'Water vapor permeability, mechanical, and structural properties of edible β–casein films', *International Dairy J*, 10, 353–358.

MAYNES, J R and KROCHTA, J M (1994), Properties of edible films from total milk protein. *J. Food Sci*, 59, 909–911.

MCHUGH, T H, AVENA-BUSTILLOS, R and KROCHTA, J M (1993), 'Hydrophilic edible films: Modified procedure for water vapor permeability and explanation of thickness effects', *J Food Science*, 58, 899–903.

MCHUGH, T H and KROCHTA, J M (1994a), 'Dispersed phase particle size effects on water vapor permeability of whey protein–beeswax edible emulsion films', *J Food Processing and Preservation*, 18, 173–188.

MCHUGH, T H and KROCHTA, J M (1994b), Sorbitol- vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation', *J Agric Food Chem*, 42, 841–845.

MCHUGH, T H and KROCHTA, J M (1994c), 'Water vapor permeability properties of edible whey protein–lipid emulsion films', *JAOCS*, 71(3), 307–312.

MCHUGH, T H and KROCHTA, J M (1994d), 'Milk protein-based edible films and coatings', *Food Technology*, January, 97–103.

MCHUGH, T H, AUJARD, J F and KROCHTA, J M (1994), 'Plasticized whey protein edible films: Water vapor permeability properties', *J Food Sci*, 59, 416–419, 423.

MI, F-L, SHYU, S-S and PENG, C-K (2005), 'Characterization of ring-opening polymerization of genipin and pH-dependent cross-linking reactions between chitosan and genipin', *J Polymer Sci*, Part A, 43, 1985–2000.

MOTOKI, M, ASO, H, SEGURO, K and NIO, N (1987), 'Alpha s1 casein film prepared using transglutaminase', *Agric Biol Chem*, 52, 993–996.

MÜLLER-BUSCHBAUM, P, GEBHARDT, R, MAURER, E, BAUER, E, GEHRKE, R and DOSTER, W (2006), 'Thin casein films as prepared by spin-coating: Influence of film thickness and of pH', *Biomacromolecules*, 7, 1773–1780.

NIEUWENHUIZEN, W F, DEKKER, H L, GRONEVELD, T, DE KOSTER, C G and DE JONG, G A H (2004), 'Transglutaminase-mediated modification of glutamine and lysine residues in native bovine lactoglobulin', *Biotechnology and Bioengineering*, 85, 248–258.

O'CONNELL, J E and DE KRUIF, C G (2003), 'β-casein micelles: Cross-linking with transglutaminase', *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 216, 75–81.

ONWULATA, C I, KONSTANCE, R P, COOKE, P H and FARRELL, H M (2003), 'Functionality of extrusion-texturized whey proteins', *J Dairy Sci*, 86, 3775–3782.

ONWULATA, C I, ISOBE, S, TOMASULA, P M and COOKE, P H (2006), 'Properties of whey protein isolates extruded under acidic and alkaline conditions', *J Dairy Sci*, 89, 71–81.

ONWULATA, C I and TOMASULA, P M (2008), 'Gelling properties of tyrosinase-treated dairy proteins', Accepted for publication to *Food Bioprocess Technol*. DOI: 10.1007/s11947-008-0124-4.

OUATTARA, B, CANH, LT, VACHON, C, MATEESCU, M A and LACROIX, M (2002), 'Use of γ–irradiation cross-linking to improve the water vapor permeability and the chemical stability of milk protein films', *Radiation Physics and Chemistry*, 63, 821–825.

OZDEMIR, M and FLOROS, J D (2004), 'Active food packaging technologies', *Critical Reviews in Food Science and Nutrition*, 44, 185–193.

PEREZ-GAGO, M B, NADAUD, P and KROCHTA, J M (1999), 'Water vapor permeability, solubility, and tensile properties of heat-denatured versus native whey protein films', *J Food Science*, 64, 1034–1037.

PEREZ-GAGO, M B and KROCHTA, J M (2000), 'Drying temperature effect on water vapor permeability and mechanical properties of whey protein–lipid emulsion films', *J Agric Food Chem*, 48, 2687–2692.

QI, P X (2007), 'Casein micelle structure: The past and the present', *Le Lait*, 87, 363–383.

RESSOUANY, M, VACHON, C and LACROIX, M (1998), 'Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films', *J Agric Food Chem*, 46, 1618–1623.

ROBERTSON, G L (1993), Processing and converting of thermoplastic polymers, in Robertson, G L, *Food Packaging, Principles and Practice*, New York, Marcel Dekker, 1–8; 111–143.

SALAME, M (1986), 'Barrier Polymers', in *The Wiley Encyclopedia of Packaging Technology*; Bakker, M, John Wiley & Sons, New York, 48–54.

SANTOS, C V, TOMASULA, P M and KURANTZ, M J (1999), 'Deamidation kinetics of casein precipitated by carbon dioxide compared with commercial caseinates', *J Food Science*, 64, 400–414.

SANTOS, C V and TOMASULA, P M (2000), 'Acylation and solubility of casein precipitated by carbon dioxide', *J Food Science*, 65, 227–230.

SCHOU, M, LONGARES, A, MONTESINOS-HERRERO, C, MONAHAN, F J, O'RIORDAN, D and O'SULLIVAN, M (2005), 'Properties of edible sodium caseinate films and their application as food wrapping', *LWT*, 38, 605–610.

SEO, S-Y, SHARMA, V K and SHARMA, N (2003), 'Mushroom tyrosinase: Recent prospects', *J. Agric. Food Chem*, 51, 2837–2853.

SHARMA, R, LORENZEN, P C and QUIST, K B (2001), 'Influence of transglutaminase treatment of skim milk on the formation of ε-(γ-glutamyl)lysine and the susceptibility of individual proteins towards crosslinking', *International Dairy J.*, 11, 785–793.

SHARMA, R, ZAKORA, M and QVIST, K B (2002), 'Susceptibility of an industrial α-lactalbumin concentrate to cross-linking by microbial transglutaminase', *International Dairy J*, 12, 1005–1012.

SHELLHAMMER, T H and KROCHTA, J M (1997), 'Water vapor barrier and rheological properties of simulated and industrial milk fat fractions,' *Transactions of the ASAE*, 40, 1119–1127.

SHELLHAMMER, T H and KROCHTA, J M (1997a), 'Whey protein emulsion film performance as affected by lipid type and amount', *J Food Science*, 62, 390–394.

SIEW, D C W, HEILMANN, C, EASTEAL, A J and COONEY, R P (1999), 'Solution and film properties of sodium caseinate/glycerol and sodium caseinate/polyethylene glycol edible coating systems', *J Agri Food Chem*, 47, 3432–3440.

SOTHORNVIT, R and KROCHTA, J M (2000a), 'Oxygen permeability and mechanical properties of films from hydrolyzed whey protein', *J Ag Food Chem*, 48, 3913–3916.

SOTHORNVIT, R and KROCHTA, J M (2000b), 'Plasticizer effect on oxygen permeability of beta-lactoglobulin films', *J Ag Food Chem*, 48, 6298–6302.

SOTHORNVIT, R and KROCHTA, J M (2000b), 'Water vapor permeability and solubility of films from hydrolyzed whey protein', *J Food Science*, 65, 700–703.

SOTHORNVIT, R, OLSEN, C W, MCHUGH, R H and KROCHTA, J M (2003), 'Formation conditions, water-vapor permeability and solubility of compression-molded whey protein films', *J Food Science*, 68, 1985–1989.

SOTHORNVIT, R, OLSEN, C W, MCHUGH, T H and KROCHTA, J M (2007), 'Tensile properties of compression-molded whey protein sheets: Determination of molding condition and glycerol-content effects and comparison with solution-cast films', *J Food Engineering*, 78, 855–860.

SOUTHWARD, C R and WALKER, N J (1980), 'The manufacture and industrial use of casein', *N Z J Dairy Sci and Technol*, 15, 201–217.

THALMAN, C R and LOTZBEYER, T (2002), 'Enzymatic cross-linking of proteins with tyrosinase', *Eur Food Res Technol*, 214, 276–281.

THARANATHAN, R N (2003), Biodegradable films and composite coatings: Past, present, and future, *Trends Food Sci Technol*, 14, 71–78.

TOMASULA, P M, CRAIG, J C JR, BOSWELL, R T, COOK, R D, KURANTZ, M J and MAXWELL, M (1995), 'Preparation of casein using carbon dioxide', *J Dairy Sci*, 78 (3), 506–514.

TOMASULA, P M, CRAIG, J C and BOSWELL, R T (1997), 'A continuous process for casein production using high-pressure carbon dioxide', *J Food Eng*, 33, 405–419.

TOMASULA, P M, PARRIS, N, YEE, W and COFFIN, D (1998), 'Properties of films made from CO_2-precipitated casein', *J Agric Food Chem*, 46, 4470–4474.

TOMASULA, P M, YEE, W C and PARRIS, N (2003), 'Oxygen permeability of films made from CO_2-precipitated casein and modified casein', *J Agric Food Chem*, 51, 634–639.

TUNICK, M and ONWULATA, C I (2006), 'Rheological properties of extruded milk powders', *International J Food Properties*, 9, 835–844.

VACHON, C, YU, H-L, YEFSAH, R, ALAIN, R, ST-GELAIS, D and LACROIX, M (2000), 'Mechanical and structural properties of milk protein edible films cross-linked by heating and γ–irradiation', *J Agric Food Chem*, 48, 3202–3209.

VARGAS, M, PASTOR, C, CHIRALT, A, MCCLEMENTS, D J and GONZALEZ-MARTINEZ, C (2008), 'Recent advances in edible coatings for fresh and minimally processed fruits', *Critical Reviews in Food Science and Nutrition*, 48, 496–511.

WHITTEN, K W, GAILE, K D and DAVIS, R E (1992), *General Chemistry*, 4th ed.; Saunders College Publishing, Harcourt Brace Jovanovich College Publishers: Philadelphia, PA.

YILDIRIM, M and HETTIARACHCHY, N S (1998), 'Properties of films produced by cross-linking whey proteins and 11S globulin using transglutaminase', *J Food Sci*, 63, 248–252.